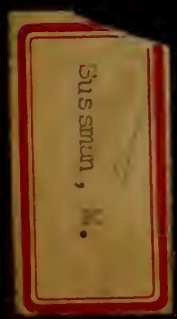

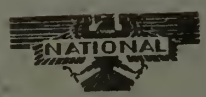

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Thesis

POISON GLANDS OF AMPHIBIA

by

Morris Sussman

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THE POISON GLANDS OF AMPHIBIA

Contents

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Section I

Amphibia in the Early Literature

From a perusal of the literature it would appear that Amphibia have been described from time immemorial. In the Talmud various types are distinguished from each other. The toad is there described as an animal whose touch contaminates and by the term tzab which means literally to swell or puff up. (Abel & Macht)(1912)

The Chinese have long used, as a remedy, a preparation derived from toadskins which they call Ch'an Su. (Chen & Chen) (1933a). Ch'an means the toad and Su the venom spelt according to the Mandarin pronunciation (Chen K.K.)(1932). This product is similar in physiological action to digitalis, but is said to be from fifty to one hundred times as powerful. Ch'an su has been used empirically in Chinese medicine for hundreds of years. It has been employed in the treatment of canker sores, sinusitis, and many local inflammatory conditions; in the relief of toothache, and in the arrest of hemorrhages from the gums. When administered internally in the form of a compound pill, it is said to be able to break colds. (K.K. Chen & A.L. Chen)(1933b). The use of toadskins as medicine in China may be more reasonable than it appears. Recent studies of the secretion of parotid glands of the toad reveal chemical changes taking place within, the matured secretion producing adrenaline. Senso, the product described

by the Japanese Shimizu (1916) is prepared in the same manner and has the same properties as Ch'an Su. The similarity in names implies a common background and one probably borrowed the process from the other.

Primitive tribes have long been familiar with the uses of poisons obtained from various kinds of tailless amphibians. In Colombia the Indians poison their arrows with a secretion from the small, brilliantly colored Dendrobates tinctorius which is less than two inches in length when fully grown. By exposing it to heat near a fire, sufficient poison may be scraped from the back of one individual to poison fifty arrows. These arrows are used by the natives especially for the shooting of monkeys.

The principal use of this same toad, however, is in "dyeing" parrots. The green and blue feathers on the head and neck of the Amazon parrot are plucked out in some fanciful pattern, and the bare skin in these areas is touched with the skin of a living Dendrobates, and again after the young feathers have begun to appear. When the feathers finally grow in, they are yellow instead of green. There is considerable demand for these artificially colored birds in the South American countries. (Abel & Macht)(1912)

Another quite dissimilar utilization of toadskins by the Orientals is in the fabrication of small articles such as purses. For this purpose the leather is admirable, being soft and thin, yet very strong, with a pleasing texture when

prepared by experts.

Peculiar superstitions exist about toads and frogs in many countries. Since most races of man observe closely only those creatures which are either directly useful to them or potentially injurious, the majority of the amphibians escaped any thing resembling close and protracted study until relatively recent years. It was not until about two centuries ago that the facts of hibernation were definitely made known to science. Before that time it was believed that frogs were procreated from the mud, an idea proposed by no less an observer than the illustrious Aristotle himself.

In Europe, in the Middle Ages, the toad was supposed to be an intimate of the alchemist and the sorcerer and to be endowed with supernatural powers. Credulous sick people were dosed with ghastly concoctions and strange brews of various inedible substances often including a toad or frog, and over which the wizard healer said weird incantations supposed to make the evil spirit depart from the body of the sick.

The toadstone was long sought by even those best informed and least credulous. The beautiful, gleaming eye of the toad was supposed to be an outward sign of the inward luster of the jewel concealed in his head. The toadstone was considered to be endowed with therapeutic qualities and to be an effective antidote for poisons. It was carried as a charm, set in a ring or worn as an amulet, or deposited on the shrine of a saint as an offering of great piety.

Boulenger (1892) quotes English literature in which the toad is described as "the 'slimy' creature that brings ill-luck to the house, spits venom, sucks the cow's udders, and after that destroys their power of giving milk".

Almost as naive is the belief, common in most parts of the United States, that the handling of toads will cause warts. While toads appear to have warts themselves, due to the swollen glands in the skin, they cannot cause warts, nor can they harm the skin in any way. After handling toads, however, care should be taken not to get any of the mucous secretion from the skin glands into one's eye or mouth, as serious trouble might ensue.

The toad is one of the most maligned of animals and not only is it gentle and harmless, but a most useful creature as well. The insect-eating habits of the tailless amphibians are of great economic value to man. Toads especially take enough food to fill the stomach completely four times in twenty-four hours, and they have been truly valuable in fighting an outbreak of sugar-beet web worms.

Man utilizes frogs' legs as food in many parts of the world. They are collected for market on a commercial scale in some fifteen States of the Union (Cochran)(1932).

At the onset of winter, near the borders of the pond, buried under logs and stones in the mud, frogs hibernate for the winter. A wise provision of Nature slows down their life processes to suit them to this complete inactivity and apparent

inanition.

In their summer activity, more than a few moments' enforced submergence in water would have drowned them. Now, in hibernation, they can pass a whole winter beneath the mud because they are not breathing. The toad and frog are even capable, when food is scarce or the weather continues unfavorable, of prolonged hibernation. Newspapers oftentimes report that on the destruction of buildings known to be decades old, a toad may at times be disclosed as having been sealed in at the time of construction, and it has been able to survive without any food for all the years. The creature proves to be as active as though it had just been disturbed after a short nap.

From the quoted information one may therefore infer that the Amphibia are not as destructive as heretofore claimed.

Section II

Types of Amphibia Studied

Class Amphibia
- Subclass 1. Stegacephali
- Subclass 2. Lissamphibia - without dermal armour
  - Order 1. Apoda - limbless amphibia
  - Order 2. Urodela - tailed amphibia
    - Family 1. Amphiumidea
      - Genus 1. Cryptobranchus
    - Family 2. Salamandridae
      - Subfamily 1. Plethodontia
        - Genus 1. Plethodon glutinosus
      - Subfamily 2. Salamandrinae
        - Genus 1. Salamandra maculosa
    - Family 3. Proteidae
      - Genus 1. Necturus maculatus
  - Order 3. Anura - tailless amphibia
    - Suborder 1. Aglossa - tongue-less
      - Family 1. Pipa americana
      - Family 2. Xenopus laevis
    - Suborder 2. Phaneroglossa
      - Family 1. Discoglossidae
        - Species 1. Bombinator igneus
        - Species 2. Bombinator pachypus
      - Family 2. Pelobatidae
        - Species 1. Pelobates fuscus
        - Species 2. Pelobates cultripes
        - Species 3. Scaphiopus
      - Family 3. Bufonidae (Toads)
        - Species 1. Bufo vulgaris
        - Species 2. Bufo marinus s. agua
        - Species 3. Bufo viridis s. variabilis
        - Species 4. Bufo calamita
        - Species 5. Bufo mauritanica s. pantherina
        - Species 6. Bufo alvarius
        - Species 7. Bufo formosus
        - Species 8. Bufo bufo gargarizans
        - Species 9. Bufo regularis
        - Species 10. Bufo arenarum
        - Species 11. Bufo viridis viridis
        - Species 12. Bufo valliceps
        - Species 13. Bufo fowleri
        - Species 14. Bufo americanus
        - Species 15. Bufo quercicus
      - Family 4. Ranidae (Frogs)
        - Subfamily 1. Raninae
          - Genus 1. Rana
            - Species 1. Rana Temporaria
        - Subfamily 2. Dendrobatinae

(continued)

Genus I. Dendrobates
Species 1. Dendrobates tinctorius

A. Urodela or Tailed Amphibia

1. Salamanders and Newts
   a. Cryptobranchus allegheniensis
      1. Cryptobranchus allegheniensis or the Hellbender is found in the eastern part of the United States.

2. Cryptobranchus japonicus, the Giant Salamander of Japan and China is similar in many respects to the American species and they shall therefore be considered together.

They both live in the water and when captured emit a peculiar slimy secretion which hardens into a gelatinous mass after a short exposure to the air. The skin displays two distinct types of glands and it is from these glands that the secretion is released. The secretion from one type is found to be granular in appearance and takes stains readily. The secretion of the other gland is only slightly affected by stains, and is not so markedly granular in appearance. The nuclei are also less numerous in this second type of gland, and are considerably larger and more rounded in shape.

   b. Plethodon (Esterley)(1904)
      1. Plethodon glutinosus, the common North American newt is found from the Ohio to the Gulf of Mexico.

a. Plethodon glutinosus s. oregoniensis has been carefully studied by Miss Ormerod. The tail of Plethodon oregoniensis is found to be enlarged both in the male and female and has nothing to do with sex.

The dorsal half of the epidermis of this organ is covered with minute and thickly crowded pores which can be seen even with the naked eye. The skin of this region is enormously

thickened and the structures prove to be greatly enlarged epidermal glands. These glands are not to be found in all species of Salamander, but are widely distributed in nature. (Hubbard)(1903)

The glands prove, on microscopic study, to be of two kinds, a granular type and a mucous type. The two are distinguished by the character and staining reaction of their secretions, and by other histological features, as well as by the size of the glands.

The bodies of the large glands possess an investing musculature, and in addition the ducts have both dilator and constrictor muscles lying in the epidermis.

The granular glands are venomous, and in the ejaculation of the secretion, the glands are destroyed. Renewal takes place by the growth into all the old glands, of a new and smaller gland which is mucous in nature. The presence of this smaller sac is not dependent upon the removal of the secretion of the large glands, for the mucous reaction is given by all the glands; in those which show no degeneration as well as in those where it is wide-spread.

The growth of the new gland is dependent upon the removal of the secretion about it. There is evidence that even in case the glands are hindered in their development, they still secrete mucous. But when not hemmed in by the heavy granular contents of the large glands, they grow, take the place and very probably assume the function of the old glands which they replace.

Both the musculature and the epithelium of the granule glands have a direct nerve supply. The gland cells are surrounded by a basket work of fibers, which in some cases have terminal expansions lying on the nuclei. The muscles are supplied by nerves with typical endings of expansions or bulbs, as well as by fine twigs without terminal expansions.

c. Salamandra maculosa, the Spotted or Fire Salamander, found over the whole of Central, Southern, and Western Europe, with the exception of isolated islands, the largest of which are the British Isles. In these salamanders the parotid glands are large and are covered with large pores. A series of distinct swellings, or cutaneous glands, each with a distinct opening, extends along either side of the back, and a shorter series along the flanks. When treated with violence, or submitted to severe pain, a milky white fluid exudes from the glands, and is, under violent contraction of the muscular skin and body, sometimes squirted out in fine jets to the distance of a foot. Burning pain and subsequent inflammation result if this venom gets into the eye. The same applies to the mucous lining of the mouth and throat. A few drops in the stomach or the blood of a small animal is sufficient to cause its death.

Many of the most venomous amphibia exhibit a conspicuous coloration similar to that of Salamandra maculosa, the combination of yellow or orange upon a dark background a widespread sign of poison.

Boulenger (1892) refers to the experiment performed by Miss Ormerod, in which she pressed part of the back and tail of a live Crested Newt between her teeth. "The first effect was a bitter astringent feeling in the mouth, with irritation of the upper part of the throat, numbing of the teeth more immediately holding the animal, and in about a minute from the first touch of the newt, a strong flow of saliva. This was accompanied by much foam and violent spasmodic action approaching convulsions, but entirely confined to the mouth itself. The experiment was immediately followed by headache lasting for some hours, general discomfort of the system, and half an hour after by slight shivering fits." This experiment shows that the poison is not septic but acts upon the heart and the central nervous system.

d. In Necturus maculosus two groups of seemingly different glands are to be found. Some are large, well-rounded and are completely filled with a dense granular secretion. Others are smaller and do not present the plump appearance of the larger glands. The general appearance of the smaller glands leads one to regard them as discharged granular glands. According to A. B. Dawson (1920), further evidence for the fact that we are dealing with two distinct types rather than one is shown by their differences in development, in histological structure, in the character and staining reactions of their secretion and in their physiological activities. Mixed glands are occasionally found. They represent stages in the replace-

ment of the mucous gland by glands of the granular type. However, what he offers as proof for the presence of two types of glands is not conclusive proof that they are distinct. In fact it seems to show that there is an interrelation between the two types of gland and that one is only a transitory stage before developing into the other.

Various authorities have studied the histology of the skin glands in poisonous Amphibia, and Bugnion in 1875 asserted that only one type of gland was really present, the granular type, and that the mucous gland is only a younger stage of the same gland. Other later workers who have confirmed his results are Leydig, Phisalix, Junius and Muhse, but this argument is outside the realm of this thesis. I shall not offer any further criticism other than that from my reading of the work done by Mrs. Muhse (1909) on the histology of the cutaneous glands of the toad, I am inclined to believe that she has offered most conclusive proof that both glands are interrelated and of a common origin.

B. Anura or Tailless Amphibia

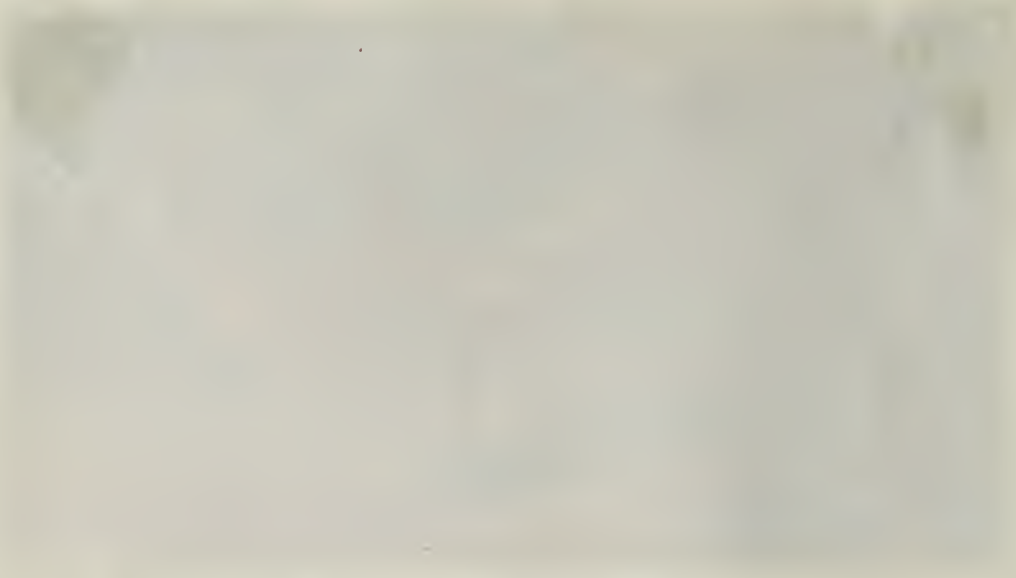
1. Aglossa or tongueless toads.

 One-half Natural Size

SUCH CLAWS OCCUR RARELY AMONG AMPHIBIANS

The Aglossa, or tongueless frogs, are here represented by the clawed *Xenopus mülleri* fro[m] [E]ast Africa. They are entirely aquatic, and hunt for their food in the mud, stirring it up with the [l]ong fingers and seeming to find it rather by touch than by sight. They must go occasionally [t]o the surface of the water for a lungful of air, which lasts them for several minutes.

a. Pipa americana or the Surinam toad is Neotropical in habitat. Examination of the skin of either sex discloses the presence of papillae which are spread over the whole surface, except on the webs of the toes, on the cornea, and on the star-shaped points of the fingers. Each papilla carries a little horny spike, and a poison-gland frequently opens near its base. Larger poison-glands exist on the dorsal sides of the body, but there are no parotid complexes. Slime-glands occur all over the surface.



THE SURINAM TOAD HAS NO FREE-SWIMMING TADPOLE STAGE

Although limited to the northeastern part of South America, where it leads an entirely aquatic existence in the pools and inundated areas, this tongueless (Aglossid) amphibian (*Pipa pipa*) has long been kept in aquaria in zoölogical gardens because of its remarkable breeding habits. At the time the eggs are laid the male distributes them evenly over the back of the female, and each egg comes to lie in a deep pouch in the skin, closed by a peculiar lid, which is probably the remnant of membrane originally surrounding the egg. Within this cavity the egg develops through the tadpole stage, and the young one, fully metamorphosed, leaves his prison and swims away from his mother to live his own independent life.

b. Xenopus laevis, the South African clawed toad is aquatic in habitat and in many respects is more like Rana, the frog, than like Bufo, the toad. From the skin, when irritated by mechanical, electrical, or chemical stimuli, a white viscid secretion exudes, mainly on the back and extensor aspects of the limbs. (Gunn)(1930)

Sections of the skin show that the secreting glands are of a simple saccular type, lined with one layer of columnar epithelium. No parotid complex of glands is here present as

in Bufo marinus s. agua and several other species of toad. The circulatory system of Xenopus laevis shows little response to the skin secretion or to adrenaline, while Abel and Macht (1912) have shown that Bufo agua reacts normally to adrenaline.

The secretion from Xenopus was obtained (Gunn)(1930) by placing the animal under a small bell-jar and slight anaesthesia was produced with ether. The secretion was then scraped away and used either at once or after being dried in vacuo. By tests on the mammalian heart, it was shown that the main active principle in the skin secretion of the South African clawed toad is a sympatho--mimetic substance similar but not identical with adrenaline. A principle which stimulates the para-sympathetic nerve-endings, was also shown as being present.

c. The fire toads, Bombinator igneus (red ventrally) and Bombinator pachypus (yellow ventrally) can be considered together because of great similarity. Bombinator igneus is found mostly in the northern part of Germany and in Russia. Bombinator pachypus is found in France and in Belgium. They both live in lakes, ponds and standing water and the secretion from their skins is poisonous and serves as a means of protection.

THESE SPECIES MAKE ATTRACTIVE AND INTERESTING PETS

In Europe we find the fire-toad or bell-toad, *Bombina bombina*, [lower] an aquatic species of primitive structure related to the True's toad of Plate III. The edible frog, *Rana esculenta*, [left] highly prized as food in Europe. A most curious example of paternal solicitude occurs in the midwife toad, *Alytes obstetricans*, [right] in which the male takes the long strings of eggs as soon as the female lays them and wraps them around his body, hopping to the water's edge to moisten daily as they require it.

d. Bombina bombina, the European bell-toad or fire-toad is found in the low-land ponds from Russia to Germany. This toad, like many other species, produces, when it is annoyed, a slimy, poisonous secretion from the glands on its back. If it cannot escape, it bends itself backward, showing the brilliant flaming color of its underparts, and likewise displays the bright undersurfaces of its hands and feet as a warning to its pursuers that it is not edible.

e. Pelobates fuscus, the spade-foot toad occurs throughout the whole of central Europe. It prefers sandy localities, since it burrows when it wishes to hide away. When captured, the skin becomes covered with a dermal secretion which com-

pares with garlic in smell, and because of this distinctive smell it has been given the name of Knoblauchskrote or garlic toad in Germany.

Other genera of spade-foot occur in Spain, Portugal and the southern and western parts of France. These are known as Pelobates cultripes and are similar to Pelobates fuscus.

 One-half Natural Size

ALWAYS ON THE QUI VIVE FOR WHAT MAY COME

True's toad, *Ascaphus truei*, [lower center and right] is not a true toad in spite of its name It belongs to a primitive and ancient family now found only in Europe, Asia and New Zealand with this lone exile in Washington State. The cold mountain brook makes an ideal home for the shy red-legged frog, *Rana aurora*, [lower left and upper right], while the Pacific tree-toad *Hyla regilla*, [upper left] is found in all Pacific Coast States.

III

The American form of the spade-foot toad, Scaphiopus is found both in North America and Mexico, and differs only slightly from the European type. Variations occur in some of the species, such as those of the southern states, Scaphiopus solitarius which have peculiar large pectoral glandular complexes, or Scaphiopus multiplicatus, the species

of Mexico has these complexes tibially.

Photograph by Lynwood M...

3. The third type or family of toad is Bufo and the first of these to be studied is Bufo vulgaris.

a. Bufo vulgaris, (Bufo bufo bufo) the Common European toad occurs throughout the Palearctic region, with the exception of Asia Minor and Ireland. It is most common in Central America and India.

The skin of the upper parts is much wrinkled and beset with numerous round warts or poison glands, which are elevated and somewhat kidney-shaped with the outlets of the ducts visible.

Phisalix and Bertrand (1893) working with the poisonous

secretion of Bufo vulgaris, found the presence of bufotalin and bufotenine, two substances with digitalis-like properties.

One-half Natural Size

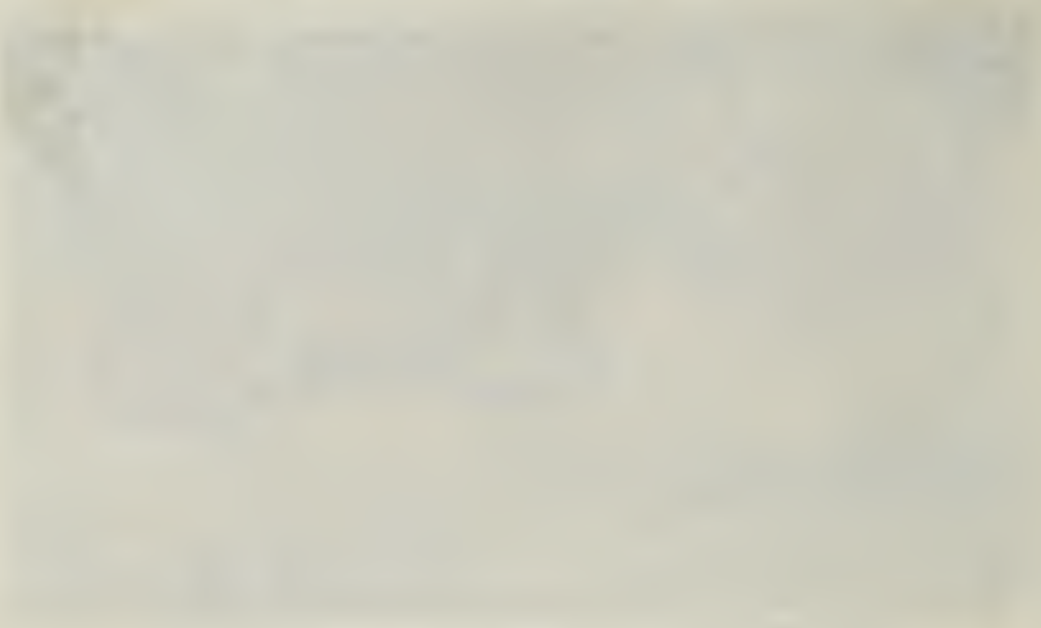
THESE ARE GIANTS IN THE TOAD FAMILY

Bufo marinus, the marine toad from tropical America, is not sea-going, as its name suggests. It is distributed over an exceedingly wide territory, however, and seems to thrive when introduced into regions where it was not previously found. In this way several of the islands of the West Indies have been populated by it, where it was brought to aid in eradicating insects harmful to agriculture.

II

b. Bufo marinus s. agua, (Abel and Macht)(1912), (Shipley and Wislocki)(1915), the species most studied and considered best experimentally, is the giant among toads. Its habitat ranges from the Antilles and Mexico to Argentina. It frequently reaches a length of eight inches, with a width of four inches. The upper parts are rough owing to the prominent warty glands, of which the parotid is enormous.

According to Gadow (1920) when the toad is treated with violence, or submitted to severe pain, a milky white fluid exudes from the glands, and is, under violent contraction



squirted out in fine jets to the distance of a foot. This phenomenon has been refuted by other workers.

OUR FRIEND THE FROG

These glands occur just behind the eye and, surrounding the upper border of the tympanum, pass backward and outward well down over the shoulders. The skin over these glands is somewhat lighter in color than that covering the surrounding tissues, and shows, even to the naked eye, the numerous pinhole-like openings of the individual gland ducts. Thus when the toad is seized in the mouth of an animal which would prey upon it, the contents of the poison glands flow freely on the animal's oral mucous membrane. (Hans Gadow)(1920)

The effect of such a charge of venom is almost immediate, burning pain and subsequent inflammation of the mucous lining of the mouth and throat, and insures the prompt release of the toad. A few drops of this poison introduced into the blood or into

the stomach of a small animal are sufficient to cause its death. Cold-blooded animals are as susceptible as warm-blooded creatures.

The parotid glands consist of simple elongated glands closely packed together, the whole bearing a resemblance to a honey-comb. The poison glands are found only on the upper surface of the body while mucous glands are to be found all over the skin, and are crowded together behind each ear. The poison glands are much larger than the mucous glands and extend deep down into the compact corium layer. They are surrounded by a thin layer of loose connective tissue which contains nerve fibers and a dense network of capillaries. There is an almost continuous layer of smooth muscle fibers about the gland. The cells of the glandular epithelium develop to an enormous size, and when mature they disintegrate, their entire plasm becoming the secretion, so that when a poison gland has reached its full development it is simply a reservoir of poison. When the poison is discharged the remains of the gland are resorbed, and at the same time one of the five or six undeveloped glands, grouped around the mouth of the functioning gland grows down alongside the remains of the discharged gland, pushing it aside to occupy its former place. (Muhse)(1909)

Mrs. Muhse (1909) found that the expulsive act is due to contraction of the smooth muscle fibers in the wall of the gland-acinus, which is under the control of the central nervous system, and the secretion is discharged in consequence of a

peripheral irritation of sufficient strength.

On histological examination, we find in the epidermis many beaker cells, each a one-celled gland, the mouth of the slender neck opening between the transitional and the molt strata. They are found in the epidermis of all regions of the body, and may occur in large numbers. They produce a granular secretion which helps loosen the molt from the underlying strata.

Cutaneous glands arise as buds in the germinating stratum of the epidermis. The bud is differentiated into neck, collar, and acinus. Large mature glands have a granular secretion which is irritating or poisonous in its effect on other animals.

The expulsion of the secretion is accomplished by the contraction of the smooth muscle fibers in the walls of the individual gland sacs. The contraction serves the double purpose of opening the duct by pulling on the radially arranged cells of the outlet, and of concerted pressure on the secretion of the lumen.

When a wart has been stimulated to exhaustion, all the gland sacs are completely emptied and most are collapsed. They then degenerate and are replaced by young gland sacs.

Madame Phisalix (1899), who studied the glands of the salamander, describes the nuclei alone as being actually engaged in the production of poison grains. It is not probable that the nuclei alone can produce the secretion, since in this case we might expect discharged glands to refill, because the naked nuclei seem uninjured in the expulsion of the secretion, and there

is no evidence that such refilling ever takes place, the emptied gland degenerating and being at once resorbed. The origin of the secretion in the glands is still unknown.

These toad glands are acted upon by the central nervous system, as their secretion is discharged in consequence of a peripheral irritation of sufficient strength.

The secretion may be obtained by squeezing the parotid glands with a curved forceps, catching the sticky secretion as it is spurted out from the numerous orifices into a large glass bowl held inverted over the toad. The poisonous secretion, according to Chen & Chen (1933), has a golden yellow color, but occasionally is milk-white or creamy in appearance. It has a fragrant smell as pointed out by Abel and Macht(1912), a slightly acid reaction, a bitter taste and causes numbness on the tongue.

The secretion can be dried in the air and used in this crude state, but preferably can be purified by grinding to a fine powder in a closed mill. It is then extracted with absolute alcohol and distilled under diminished pressure. The residue is extracted with chloroform and then evaporated and poured drop by drop into a large volume of petroleum ether. The resinous precipitate thus obtained is taken up by a small amount of chloroform and again added to an excess of petroleum ether. Finally it is allowed to crystallize out in a fairly pure state.(Chen, Jensen & Chen)(1931)

As a result of the study of Chen & Jensen (1928-9) six

principles were separated from the secretion and subjected to pharmacological tests. The chemical substances obtained from the dried venom are cholesterol, cinobufagin, cinobufotoxin, cinobufotenine, suberic acid and adrenaline.

1. Cholesterol, as obtained from dried venom in the impure state, contains ergosterol, which is responsible for the antirachitic property. The amount of ergosterol present in the cholesterol varies from 0.0 to 0.1% according to the source of the material and the method of purification. Ergosterol, irradiated with ultraviolet rays, becomes vitamin D, the preventive of rickets.

2. Cinobufagin found in toad venom has an action similar to that of digitalis, powerfully stimulating the heart, and in more than smallest doses producing serious consequences. Cinobufagin makes up 36% of the venom, is soluble in chloroform and acetone, and fairly soluble in absolute alcohol.

Cinobufagin causes a local anaesthetic action on the mucous membrane of the tongue and lips and produces numbness. Cinobufagin is used to lessen the sensation of pain. It raises blood pressure and stimulates the isolated intestines and uterus. It exerts a vasovonstrictor action when an aqueous solution is applied to the conjunctival sac, it causes vasoconstriction of the heart. Its powerful stimulating action on the cardio-inhibitory center classes this drug with the most effective members of the digitalis series.

The effect of cinobufagin may be shown experimentally on

the mouse. An hypodermic injection of the drug produces immediately a rapid rate of respiration and restlessness. In most instances the animal soon shows incoordination of movements. Finally the animal lies on its side, respiratory movements become gradually shallower and slower, but the animal soon recovers. A large dose of the drug causes violent dyspnoea and fatal convulsions. The same reaction is obtained with a rabbit as with a mouse.

In the dog and cat, when administered hypodermically or through the mouth, the drug causes, after a short time, nausea and vomiting accompanied by rapid respiration, which finally assumes the character of Cheyne-Stokes' respiration. An autopsy immediately following death discloses the heart contracted in systole.

It is evident that this substance is a heart poison. However, some respiratory disturbances and medullary stimulating effect in frogs may in all cases be observed, besides the effects on the heart. Cinobufagin was first isolated by Abel and Macht (1911-12) from Bufo marinus and later from Bufo formosus by Kotake (1928) and by Wieland H. and Vocke F. (1930).

3. Cinobufotoxin is similar to cinobufagin in heart action, causing a digitalis-like effect, but it has no local anaesthetic action. Ten minutes after injection of cinobufotoxin in dosage of 0.01 to 0.02 mgm. per gram into the anterior

lymph sac, the frog often shows a little restlessness, followed by weakness in the legs so that the animal lies flat on its abdomen. It appears spastic and stuporous, and fatal doses cause death at systole. It is a convulsant poison which is to be classed as a member of the picrotoxin group. It has been shown experimentally that the drug acts directly upon the medulla oblongata. (Chen, Chen & Jensen)(1932). Weiland and Alles (1922) first isolated bufotoxin from the skin of Bufo vulgaris. Chen, Jensen and Chen (1932) later obtained the ingredient from ten additional species of toads.

Both cinobufagin and cinobufotoxin have a direct action on smooth muscle fibers (Chen, Jensen and Chen)(1931) and in their pharmacological actions are quite similar to that of the digitalis glucosides. (Jensen and Chen)(1930)

Because of the local anaesthetic property of cinobufagin it is recommendable in the treatment of tooth-ache and canker sores.

4. Cinobufotenine which has been isolated by the Chens and Jensen from the dried venom of the Chinese Toad resembles chemically and physiologically the bufotenine obtained by Handovsky from the common European Toad (Bufo vulgaris).

Cinobufotenine dissolves in hot water from which it crystallizes out, on cooling, in clusters of fine long needles which are soluble in alcohol. A 0.02% solution in Ringer's solution is used for physiological experiments.

When tested on the heart of a frog the first result is

an increase in the tone of the heart muscle, the heart rate diminishes and the amplitude of the contractions becomes smaller. It causes a contraction of smooth muscle and a resultant rise in blood pressure, similar in effect to adrenaline, but cinobufotenine causes a constriction of the pupil, an effect opposite to that of adrenaline.

5. Suberic acid has been found in the toad venom but it is a less active substance than the other ingredients present. It has been shown to inhibit the contractions of isolated strips of intestine and uterus. (Jensen and Chen)(1930)

6. Epinephrine or adrenaline is the most important of the chemical substances present and on comparison with vertebrate suprarenal gland secretion is found to be similar.

The presence of adrenaline in the poisons of several species of toads is of unusual interest. First, it is secreted in the skin glands, an organ totally different from the suprarenal glands. Secondly, these skin glands have well formed ducts from which the secretion, including adrenaline, finds its exit, so that adrenaline in this instance cannot be termed a hormone of a ductless gland.

A chemical test to prove the presence of adrenaline in the venom is performed by adding ferric chloride to a solution of the poison and obtaining a green color. The fact that such a solution turns pink when exposed to air, and exerts a powerful vasoconstrictor action, shows that it is closely allied to, if not identical with, the adrenaline of the supra-

renal glands.

Adrenaline acts promptly as a vasoconstrictor when applied to the blood-vessels of the toad from which it is derived. (Chen & Chen)(1933). The animal has not acquired an immunity against this poison. The constriction of the blood vessels causes an increase of blood pressure, On intravenous injection into a mammal the bladder is inhibited, the uterus contracts, the stomach and gall bladder inhibited, and the bile secretion increased. Peristalsis of the intestine may be inhibited and the tone of the muscles relaxed by the injection of the toad venom. Pressor action, vasoconstriction, bronchodilation, inhibition of the isolated intestines and increase in blood sugar are all typical epinephrine reactions.

The various results obtained by chemical analysis, by the use of the polarimeter, by quantitative and qualitative physiological experiments have demonstrated conclusively that the substance isolated from the poison glands of Bufo marinus is identical with that produced by the suprarenal glands of the higher animals.

The use of adrenaline in the treatment of diabetes is invaluable. The great possibilities of toad venom in the field of medicine seem paramount therefore when one understands the possibility for an unlimited supply from this source.

The venom when applied to the eye causes a marked dilitation of the pupil with its consequent interference with accommodation. The dilitation of the pupil is due to the pre-

sence of adrenaline in the venom.

Helene Mastl (1927) found no sex difference observable in the effect of adrenaline on blood pressure. By examining the pressor response to small doses of adrenaline in cats, the author, however, showed that it is higher in males than in females. With increasing concentrations of adrenaline, the height of muscle contraction progressively decreases.

The adrenaline present in the secretions of several species of toads does not, as in higher mammals, appear to circulate in the blood stream or to play an important part in the functioning of the body. (Chen & Chen)(1933)

Phisalix and Bertrand (1893) claimed to have found toad venom in the blood in sufficient quantity for biological assay. Chen, Chen and Jensen (1932), however, have refuted their work and have shown that the adrenaline of the skin glands does not enter the blood stream.

Lutz (1933) found the pressor threshold for Bufo marinus (an amphibian) to adrenaline equal to that obtained by Wyman and tum Suden (1932) with normal rats (mammals).

c. Bufo viridis s. variabilis, the Green or Variable Toad is usually about three inches long and is the prettiest toad of Europe. It has well-developed parotid glands and a similar pair of glands sometimes occurs in the inner side of the calf, especially in the Central Asiatic and the Algerian specimens.

d. Bufo calamita or the Natterjack is the popular representative of the Green Toad in Western Europe. Small parotid glands are present plus a similar pair of glands which lie on the upper surface of the fore-arm and another on the calf. Natterjacks when caught become covered with a foamy lather, the exudation of their glands which has a peculiar smell, reminding some people of gunpowder, others of india-rubber.

e. Bufo mauritanica s. pantherina, the Pantherine Toad is one of the few African species. The parotid glands are large and flat. Bufo regularis is another representative of the Pantherine toad in Africa.

The action of the parotid gland secretion of Bufo regularis was studied and the presence of adrenaline-like and digitalis-like bodies was demonstrated. (Epstein and Gunn) (1930). The adrenaline effect was tested on the isolated intestine of the cat and of the rabbit. Acceleration and augmentation of the heart-beat plus inhibition of the intestine were the main adrenaline effects noted.

The tightly contracted pale ventricle and the dilated auricles seen in frogs which have received lethal doses of secretion are factors which imply the presence of a digitalis-like substance. Similar affects in the perfused heart of the frog and the mammal; the central vagus slowing, the heart block, fibrillation and other irregularities of the heart, and the contraction of plain muscles, e.g., the intestine, are all factors which show the presence of a digitalis-like substance.

TOADS ARE "NICE PEOPLE"

This conviction is deepened upon meeting *Bufo alvarius*. The group is as bland and philo-
ophical as old gentlemen about to take up golf. Although they live in Arizona and in southern
lifornia, reputedly one of our most arid regions, these toads are by choice semi-aquatic. They
y near water-holes in canyons, and irrigation ditches are a boon to them.

The animal, however, showed a high degree of tolerance for digitalis and also for its own secretion.

f. Bufo alvarius, the Colorado River toad, attains the distinction of being the largest toad found within the borders of the United States, its head-and-body length being more than five inches. Its smooth greenish skin causes it to resemble a frog more than a toad, while its semi-aquatic habits heighten the similarity. The skin is moist and slippery not unlike that of frogs. (Cochran)(1932)

Bufo alvarius is unique in that in addition to the pair of parotid glands, it has a pair of glandular swellings with definite outline on the forearms, another pair on the thighs, and three pairs on the legs. These glands afford the animal an unusual degree of protection, for their secretion is highly poisonous, and has been known to cause immediate death to a dog which rashly molested one. Only great stress or fear, however, causes the poison to be released from these glands, for captive specimens gently handled never display the slightest trace of it, even when they are forcibly fed.

The secretion, expressed from the parotid glands and other glandular swellings, is creamy white, acid to litmus, imparts to the tongue a bitter taste, and has a peculiar smell not unlike that of an over-boiled egg.

g. Bufo formosus is a large Japanese toad comparable to Bufo marinus in size. Its skin is brownish to olive-green with a bluish hue. Some specimens have a reddish-brown tint over

the head. A thin straight light brown streak runs from the cephalic to the caudal end. In addition there are two light colored streaks, one on each side of the back, running lengthwise on the glands and extending to the flanks. The warts are blunt and greenish-blue in color.

The expressed secretion from the parotid glands is milky white, odorless, acid to litmus, and bitter to the taste, causing numbness of the tongue in a few minutes.

h. Bufo bufo gargarizans is a Chinese species of toad and has been studied in an effort to determine the identity of the toad from which Ch'an Su is prepared. Its skin is dull green and the warts are prominent and pointed. The tips of the warts are colored black. The parotid glands are prominent, raised, bean-shaped, and covered by a greenish black integument. The outlets of the ducts of the poison glands are definitely noticeable. The expressed secretion from the parotid glands is milky white, acid in reaction, has a peculiar grassy smell, and is bitter to the taste, causing numbness of the tongue and lips.

It may be interesting to point out that the use of Ch'an Su in China for toothache, sinusitis, and bleeding of the gums appears to have a good foundation. Cinobufagin has a local anaesthetic action and adrenaline is employed in modern medicine for rhinitis and small hemorrhages. In other words, the Chinese empirically recognized the astringent and hemostatic properties of adrenaline, and appreciated the local anaesthetic action of cinobufagin long before any scientific investigations

were undertaken.

The exact method of manufacturing Ch'an Su has been kept secret among Chinese druggists. However it has been indirectly disclosed that the poisonous secretion of the Chinese toad, Bufo bufo gargarizans, is the source of Ch'an Su. (Chen & Chen)(1933d)

i. Bufo regularis, the South African toad has been studied by Epstein and Gunn (1930). The dorsal skin has an olive-green background with large black or brownish-green patches which are almost symmetrically arranged on each half of the body. Every patch on the back has a distinct brownish-yellow halo. A thin streak of the same color runs from the cephalic to the caudal ends along the vertebral column. Small glands occur on the back and on the legs. The parotid glands are prominent and somewhat kidney-shaped, with visible orifices of the glandular ducts. The secretion expressed from the parotid glands is creamy, acid to litmus, practically odorless, and bitter to the taste, producing numbness of the tongue.

j. Bufo arenarum is a South American species. Its dorsal skin is brownish-green with patches of lead-blue. The warts are prominent and have a black point. The parotid glands are elongated. The expressed secretion from the glands is milky white, has a grassy smell, is acid to litmus, irritating to the nose, and bitter to the taste and produces numbness of the tongue.

k. Bufo viridis viridis is the European green toad. The

dorsal skin of this species has a brownish-gray background, tinged with a bluish-green cast. There are irregular patches of deep chrome-green cast, which have a black margin, and a halo outside of it. Although the skin is comparatively smooth, the warts can be definitely made out. ~~There are orange colored dots in the center of the warts which occur on the flanks~~ The warts on the flanks have orange colored centers. The outline of the parotid glands can be made out only by careful examination. They are oval in shape and slightly raised. The orifices of the glandular ducts can be readily noted. The expressed secretion from the parotid glands is milky white, acid to litmus, has a peculiar grassy smell, is bitter to the taste, and produces numbness of the tongue and lips.

1. Bufo valliceps is a species of toad indigenous to the United States. Its dorsal skin has a brown background with large black patches. The head is slightly olive-green. Three distinct streaks appear on the back, one over the vertebral column, and two on the sides extending to the flanks. There are numerous small warts over the back and limbs. The parotid glands are comma shaped, and yield a secretion, which is yellowish white, acid to litmus, and bitter to the taste, produces numbness of the tongue and has a grassy smell.

m. Bufo fowleri is another North American species. The dorsal skin has a light olive-green background with greenish-black patches. There is a yellowish-green halo around each patch. The warts are pointed, small, and have dark brown tips. The parotid glands are oval in shape, but in large toads they may have a short, blunt, lateral projection. The secretion expressed from the parotid glands is grayish-white, sticky, leathery, grassy in odor, acid to litmus, and bitter to the taste, producing numbness in the tongue.

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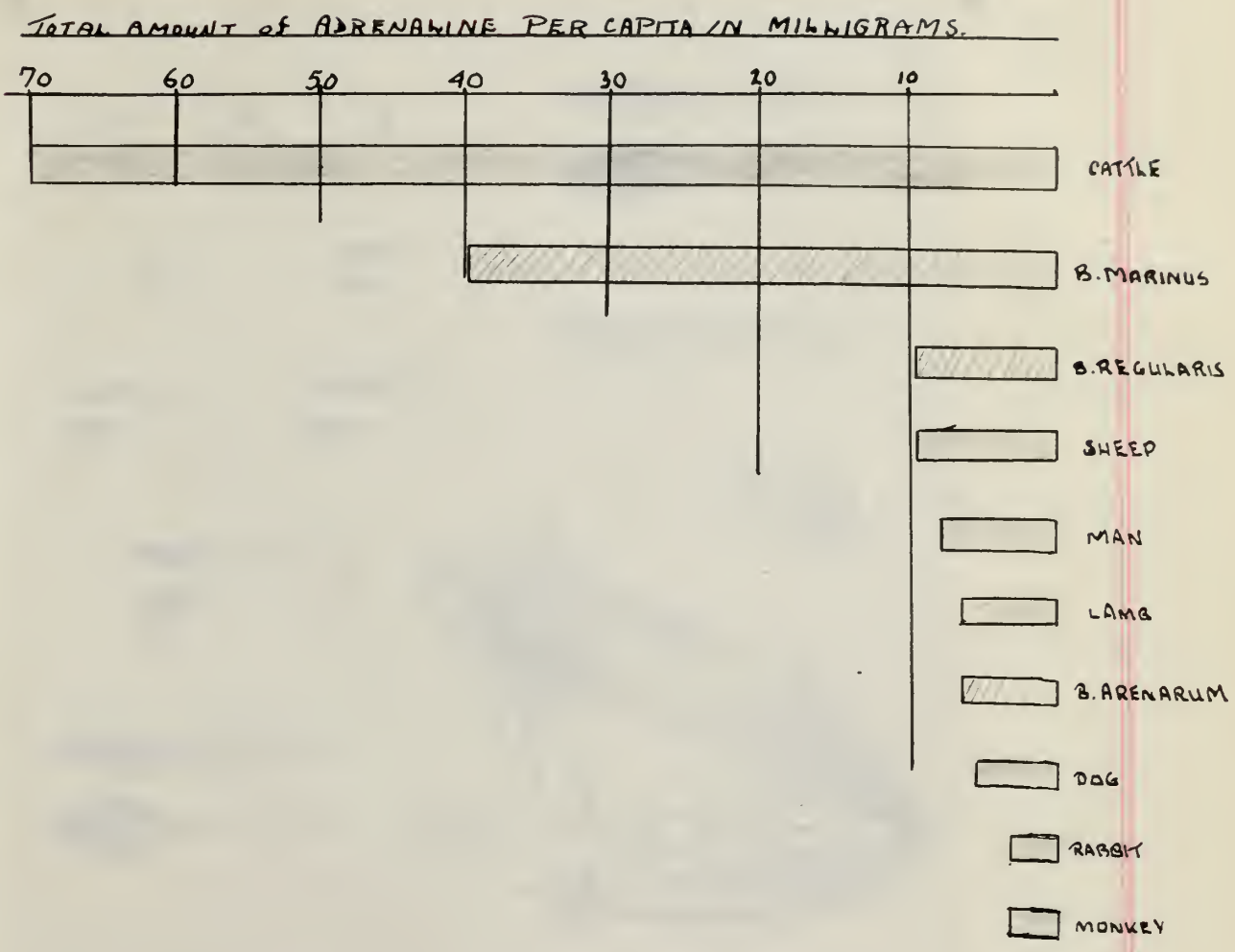
THE AMERICAN TOAD (BUFO AMERICANUS) TRILLING HIS EVENING SONG.

n. Bufo americanus, the common American toad is similar in general appearance to Bufo fowleri. Bufo americanus is darker, its patches on the back usually enclose a single wart, and the warts are more prominent and larger, whereas the Fowler toad is paler, its patches often enclose three or more warts and the warts are smaller. The parotid glands are elevated, oval in shape, and have visible orifices. The secretion expressed from these glands is grayish-white, sticky like rubber cement, somewhat grassy in smell, acid to litmus, and bitter to the taste, producing numbness of the tongue.

o. Bufo quercicus is one of the smallest species of North American toad. The dorsal skin has an olive-green background with black patches on the back and legs. Warts are generally distributed and have brown tips. A distinct greenish-yellow streak runs over the vertebral column. The parotid glands are oval-shaped. The secretion expressed from these glands is creamy white, acid to litmus, and has a peculiar odor similar to that of the secretion of Bufo viridis viridis.

On comparison with total quantities of adrenaline in the higher mammals as determined by Folin, Cannon, and Denis (1912-13) and by Elliott (1913), the Jamaican toad, B. marinus stores in its parotid glands on the average a total quantity of adrenaline which is more than half that in a pair of cattle suprarenal glands, or more than four times the amount present in a pair of human suprarenal glands. The South African toad, B. regularis, has the same amount as a sheep, and B. arenarum

the same amount as a lamb.



FOLIN, CANNAN AND DENIS (1912-13)

Drawing by Hashime Murayama

THE LIFE HISTORY OF THE FROG

The development of the wood-frog, *Rana sylvatica* (Plate III), is outlined here in various stages, from the egg to the fully adult form: (A) first day, (B) second day, (C) fourth day, (D) fifth day, (E) eighth day, (F) tenth day, (G) twelfth day, (H) fourteenth day, (I) third week, (J) seventh week, (K) beginning of ninth week, (L) eleventh week, (M) third year. One and one-half times natural size. In the preparation of this drawing and the paintings from life (pages 635-642) the National Geographic Society is indebted to Dr. William M. Mann, Director of the National Zoölogical Park, for helpful coöperation.

5. Rana - Frogs (Holmes)(1911)

The skin of the frog, like that of most of the Amphibia, is richly furnished with glands. A few species have a pair of large flat glands at the base of or on the inner side of the arms. These species are; Rana glandulosa of Borneo, Rana temporalis

of Ceylon, Rana elegans of West Africa, and Rana albolabris of West Africa. These glands are of the simple alveolar type, and lie mainly in the stratum spongiosum of the corium. Only rarely do they extend into the deeper portions of the skin.

Two varieties of gland are commonly distinguished which may be designated as the mucous glands and the poison glands.

The mucous glands are smaller much more abundant than the poison glands, and are fused over practically the entire surface of the body. In some places they are so thick that they nearly touch. Their ducts are narrow and are lined with a layer of small flattened epithelial cells. The body of the gland is lined with epithelial cells which form a single layer except near the opening of the neck, where there are two layers. It is this epithelium which forms the mucous that is discharged into the lumen of the gland and poured out through the neck over the surface of the skin.

Outside of the epithelium is a muscular coat composed of smooth muscle cells which lie in a meridional direction. The outermost coat of the gland is formed by a layer of fibrous connective tissue. The function of the muscle cells is the expulsion of the secretion of the gland. The glands of the skin are in constant motion, ~~---~~ changing not only in size, but also in form, being now rounded, now wrinkled and angular. Contraction may be caused by stimulation of the skin with irritant solutions or by electric current.

The poison glands are larger and less abundant than the

mucous glands, and less uniformly distributed over the surface of the body. They are more numerous on the dorsal side of the body and are especially abundant, and unusually large in the lateral dermal plicae. Like the mucous glands they possess a muscular and connective tissue coat outside the layer of epithelium. The chief difference in the two types of glands, with the exception of size and thickness of the tunics, lies in the secreting cells. Englemann described the epithelium as consisting of cylindrical cells nearly filled with granules.

The secretion of the poison glands is a whitish fluid with a burning taste.

a. Rana temporaria, the common European Brown Frog or Grass-frog has a smooth skin which is always moist owing to the minute mucous glands. A series of larger glands forms a pair of folds along the upper sides of the back. Beginning behind the eyes, they converge slightly beyond the shoulders, diverge a little in the sacral region, and converge again towards the ventral.

b. Dendrobates tinctorius is usually found from Panama to Ecuador and to the mouth of the Amazon. It is about one and one-half inches in length. This species owes its specific name to the peculiar use made by man of the strongly poisonous secretion of the tiny glands of the otherwise smooth skin. Other species are doubtless employed in the same way. The poison is mainly used for "dyeing" the green Amazon parrots as described in the introduction to this thesis.

THE IRIDESCENT BEAUTY OF FROGS AND TOADS

c. Rana pipiens, the Leopard-frog is found east of the Rocky Mountains over North America into Mexico. Especially prominent in these species are the lateral glandular folds, one on each side of the back, from which originates the secretion of slime to lubricate the skin. Since cutaneous respiration assists the lungs, and in some species entirely replaces them, it is necessary that the surface of the body be kept moist at all times.

Section III

A Comparison of Certain Pharmaceutical Drugs With Amphibian Poisonous Secretions

From a study of the amphibian poison, both from a chemical and physiological aspect the most active drugs are adrenaline and digitalis-like substances. It is therefore appropriate to turn to a study of these pharmaceutical drugs and compare their properties with the substances isolated by Abel and Macht (1912) and by Chen and Jensen(1928-9).

According to Chen and Chen (1934) the substance adrenaline is present in the secretion of five species of toads. From the poison of B. marinus, B. regularis, B. arenarum, B. formosus and B. bufo gargarizans, adrenaline has been obtained.

The physiological properties of the amphibian cutaneous secretion have been tested by innumerable workers, the outstanding amongst whom may be listed Vulpian (1854), Abel and Macht (1911-12), Epstein and Gunn (1930) and Chen, Jensen and Chen (1931).

Since Vulpian's discovery (1856) that the toad is resistant to its own poison, attempts have been made to determine the quantitative ratio between the susceptibility of the toad and of the frog. Abel and Macht (1911) reported the sensitivity of the toad's blood vessels to adrenaline. Chen and Chen (1935) found that the toad's heart, as compared with the frog's, is resistant to the action of bufagin and bufotoxin

which have a digitalis-like action. The toad's heart is, however, equally as sensitive as the frog's heart to adrenaline and bufotenine. That the toad is immune to its own poison, as Abel and Macht and other early workers believed, is therefore not entirely correct.

The typical actions of the drug adrenaline consist in a highly specific stimulation of the receptive mechanism of the entire sympathetic system. (Sollman)(1932). The effects upon any given organ whether augmentory, inhibitory, or indifferent, therefore correspond with the effects of stimulation of its sympathetic innervation. Very dilute concentrations may have opposite effects.

The most important practical manifestation of this action consists in a high rise of blood pressure, from peripheral stimulation of the vasoconstrictor mechanism of the systemic vessels, and of the accelerator mechanism of the heart.

Adrenaline is utilized especially in cases of acute arrest of the previously healthy heart. A condition that may have been induced by anaesthesia or asphyxia, especially in the new-born. The chance of revival diminishes rapidly however, and permanent success is scarcely possible if the circulation has stopped longer than ten minutes. An intravenous injection of adrenaline has thefollowing effects:

1. Rise of blood pressure due to vasoconstriction of the blood vessels.
2. Inhibition of the bladder, stomach and gall bladder.

3. Contraction of the uterus
4. Increased bile secretion, salivation and lachrymation.
5. Inhibition or stimulation of the internal anal sphincter.
6. Mydriatic effect on the eye.
7. Peristalis in intestine inhibited and tone relaxed.

The digitalis-like action of amphibian venom is due to the two substances, bufagin and bufotoxin, either separately or together. According to Sollman(1932), digitalis produces the following important reactions:

1. Increased contractility of the cardiac muscle i.e. lowered threshold of irritability.
2. The tone of the heart muscle is increased so that the systolic shortening becomes greater and more prolonged.
3. The vagus-center is stimulated.
4. Digitalis acts directly on the blood vessels but due to complicated inter-actions the results obtained are often contradictory.

Digitalis is highly useful in the course of chronic diseases, when the heart contracts imperfectly and becomes dilated; either because of incoordinated or irregular contractions, as in auricular fibrillation; or because of exhaustion of the cardiac muscle by its inability to compensate for the mechanical defects of structural lesions. The indications for its employment are governed by the functional conditions of the cardiac muscle, and only indirectly and to a minor degree by the

gross anatomic lesions. (Sollman)(1932)

DIGEST

Amphibia are referred to in the early literature but no careful study was made until 1854 when Vulpian demonstrated that toad venom has a digitalis-like effect. Later investigators directed their search more for those principles which have a specific action on the heart. Phisalix and Bertrand (1893), working with the poisonous secretion of Bufo regularis, found the presence of bufotalin and bufotenine. A distinct advance in the knowledge on toad poisons was made by Abel and Macht in 1911 when they isolated crystalline adrenaline and bufagin from the parotid secretion of Bufo marinus. They proved that bufo-adrenaline has a pressor action similar to that of the adrenaline obtained from mammalian suprarenal glands, and that bufagin has definitely a digitalis-like action.

The Chinese empirically recognized the astringent and hemostatic properties of adrenaline and appreciated the local anaesthetic action of cinobufagin long before any scientific investigations were undertaken.

Since 1928 Chen, Jensen and Chen have been engaged in a chemical study of the principles present in the dried venom of Bufo marinus. The substances isolated by these workers are adrenaline, cinobufagin, cinobufotenine, cinobufotoxine, cholesterol and suberic acid.

Cinobufagin and cinobufotoxine have a digitalis-like effect and are responsible either separately or jointly for the toxic effect produced by the various amphibian parotid glands.

Chen and Jensen (1928-9) have shown that adrenaline is present in the secretions of five species of toads. The toads are Bufo regularis, Bufo marinus, Bufo arenarum, Bufo formosus and Bufo bufo gargarizans.

Bufo marinus, the giant among the toads, stores in its parotid glands a quantity of adrenaline which is more than half that in a pair of bovine suprarenal glands, or more than four times the amount present in a pair of human suprarenal glands. With this knowledge before them, and aware that the adrenaline in the parotid gland of the toad is fairly pure, Jensen and Chen have been using the venom of Bufo marinus. Experimentally they have analyzed the active constituents of the substance with an eye toward the synthetic preparation of the active ingredients on a commercial scale.

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